

Application No.: 09/973,088  
Amendment Dated 29 March 2006  
Reply to Office Action of 29 December 2005

*AMENDMENTS TO THE SPECIFICATION*

Please amend paragraph [0116] to read as follows.

[0116] In this example, transformation, selection, and eradication experiments were conducted using somatic embryogenic cell lines from five different *Pinus radiata* families wherein a standard commonly-used somatic embryogenesis process was followed and, by making only the changes taught in the method described in this application in the preceding examples, transgenic *Pinus radiata* was produced. In the above examples, the media described in cited U.S. Patents as being sufficient to promote growth and embryogenesis of southern yellow pines and hybrids were adapted by our method to create media for the purposes of eradicating *Agrobacterium* and selecting transformants. In the present example, the maintenance media described in U.S. Patent 5,565,355 (which is hereby incorporated by reference) as being sufficient to promote growth of *P. radiata* are adapted by our improved method to create preparation, recovery, selection, and eradication media for the purposes of transforming *P. radiata* somatic embryogenic cells with *Agrobacterium*, eradicating *Agrobacterium* and selecting transformants. These examples serve to illustrate that any nutrient media that have been established as sufficient to promote growth or embryogenesis of the target tissue may be employed in conjunction with the present method without undue experimentation. The maintenance medium of U.S. Patent No. 5,565,355 is:

Standard Embryogenesis Medium (embryogenic tissue maintenance medium):

Major ion stock	40 ml
Minor ion stock	20 ml
Iron chelate stock	20 ml
Vitamin stock	10 ml
Inositol	1.0 gm
Sucrose	30.0 gm
Difco Bacto agar	8.0 gm

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(Adjust pH to 5.6-5.8 before addition of agar and autoclaving. Add filter sterilized amino acids after autoclaving.)

Major Ion Stock (make up to 400 ml):

Compound	Weight (gm)
KNO <sub>3</sub>	14.31
MgSO <sub>4</sub> •7H <sub>2</sub> O	4.00
CaCl <sub>2</sub> •2H <sub>2</sub> O	0.25
NaNO <sub>3</sub>	3.10
NH <sub>4</sub> H <sub>2</sub> PO <sub>4</sub>	2.25

Minor Ion Stock (make up to 200 ml):

Compound	Weight (mg)
MnSO <sub>4</sub> •4H <sub>2</sub> O	36.0
H <sub>3</sub> BO <sub>3</sub>	80.0
ZnSO <sub>4</sub> •7H <sub>2</sub> O	250.0
KI	10.0
CuSO <sub>4</sub> •5H <sub>2</sub> O	24.0
Na <sub>2</sub> MoO <sub>4</sub> •2H <sub>2</sub> O	2.0
CoCl <sub>2</sub> •6H <sub>2</sub> O	2.0

Iron Stock (make up to 1 liter):

FeSO <sub>4</sub> •7H <sub>2</sub> O	1.5 gm
Na <sub>2</sub> EDTA	2.0 gm

Vitamin Stock (make up to 1 liter):

<u>Thiamine HCl</u>	<u>0.5 gm</u>
<u>Nicotinic Acid</u>	<u>0.5 gm</u>
<u>Pyridoxine HCl</u>	<u>0.05 gm</u>

Amino Acids:

amino acid	amount
glutamine	110 mg/L
asparagine	105 mg/L
arginine	35 mg/L

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minor amino acids stock                  2 ml/L

Minor Amino Acids Stock (make up to 800 ml):

Amino Acid	weight (gm)
citrulline	1.58
ornithine	1.52
lysine	1.10
alanine	0.8
proline	0.7

(Dispense into 40 ml aliquots. Freeze immediately, store frozen, and thaw only on day of use. Adjust pH to 5.6-5.8 and filter sterilize before use.)